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# Potential Bronchoconstrictor Stimuli in Acid Fog

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**Acid fog is complex and contains multiple stimuli that may be capable of inducing bronchoconstriction. These stimuli include sulfuric and nitric acids, the principal inorganic acids present; sulfites, formed in the atmosphere as a reaction product of sulfur dioxide and water droplets; fog water itself, a hypoosmolar aerosol; the organic acid hydroxymethanesulfonate, the bisulfite adduct of formaldehyde; and gaseous pollutants, e.g., sulfur dioxide, oxides of nitrogen, ozone. Given this complexity, evaluation of the respiratory health effects of naturally occurring acid fog requires assessment of the bronchoconstrictor potency of each component stimulus and possible interactions among these stimuli. We summarize the results of three studies that involve characterization of the bronchoconstrictor potency of acid fog stimuli and/or their interaction in subjects with asthma. The results of the first study indicate that titratable acidity appears to be a more important stimulus to bronchoconstriction than is pH. The results of the second study demonstrate that sulfite species are capable of inducing bronchoconstriction, especially when inhaled at acid pH. The results of the third study suggest that acidity can potentiate hypoosmolar fog-induced bronchoconstriction.**

## Introduction

Complex mixtures of atmospheric pollutants occur commonly throughout the United States, particularly in areas where acidic pollutants mix with ambient fog. Naturally occurring fog has recently been shown to be quite acidic, with pH values as low as 1.7 (*1*). The major ions present in acid fog are hydrogen, sulfate, nitrate, ammonium, and chloride (*1,2*), suggesting that the low pH is in large part due to the presence of sulfuric ($H_2SO_4$) and nitric ($HNO_3$) acids. The buffering capacity of naturally occurring acid fogs has not been adequately measured. However, the presence of weak organic acids may allow an increase in the total titratable acidity of such fogs at any given pH. The potential adverse health effects of breathing acid fog have not been adequately characterized.

Acid fog contains multiple stimuli that may be capable of inducing bronchoconstriction. These stimuli include $H_2SO_4$ and $HNO_3$, the principal inorganic acids present; sulfites, formed in the atmosphere as a reaction product of sulfur dioxide ($SO_2$) and water droplets; fog water itself, a hypoosmolar aerosol; the organic acid hydroxymethanesulfonate, the bisulfite adduct of formaldehyde; the airway cooling capacity of fog droplets that are cooler than body temperature; and gaseous pollutants (e.g., $SO_2$, oxides of nitrogen, ozone). Given this complexity, evaluation of the potential bronchoconstrictor effects of naturally occurring acid fog first requires assessment of the mechanisms of action of each component stimulus and then requires examination of possible interactions among these stimuli. Mechanisms of action of component stimuli and initial characterization of interactions between stimuli are easily studied under artificial conditions (i.e., mouthpiece exposures) that allow one to examine several doses of the stimulus of interest and to more tightly control stimuli that are not of interest. We report here, in summary fashion, the results of three studies that involve characterization of the bronchoconstrictor potency of acid fog stimuli and/or their interaction.

## Role of Titratable Acidity in Acid Fog-Induced Bronchoconstriction

The first study directly examined the significance of acidity itself as a bronchoconstrictor stimulus (*3*). We hypothesized that buffered acid fogs (with a greater available pool of hydrogen ions) would cause more bronchoconstriction than unbuffered acid fogs at the same pH. Because the airway lining fluid has a con-

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siderable capacity to buffer acid, we reasoned that inhalation of buffered acid would cause a more persistent decrease in airway pH. If a change in pH is the primary mechanism by which bronchoconstriction occurs following inhalation of acid fog, then buffered acids should have more potent bronchoconstrictor effects than unbuffered acids.

Fogs of hydrochloric acid (HCl) and $H_2SO_4$ in an unbuffered state and buffered with glycine at pH 2 were administered to eight nonsmoking subjects with mild asthma. The buffered acids were given in order of increasing titratable acidity [defined as the number of milliliters of 1N sodium hydroxide (NaOH) required to neutralize 100 mL of acid solution to pH 7]. Each set of buffered or unbuffered acid fogs was given on a separate day and each fog was inhaled through a mouthpiece during 3 min of tidal breathing. A dense fog (liquid water content approximately 90 $g/m^3$) was generated by an ultrasonic nebulizer that produced particles in the large respirable size range [mass median aerodynamic diameter (MMAD) 5.3–6.2 μm]. Bronchoconstriction was assessed by measurement of specific airway resistance ($SR_{aw}$) before and after inhalation of each fog.

The subjects' asthma remained stable throughout the study and there were no significant deviations in baseline $SR_{aw}$. $SR_{aw}$ increased by more than 50% above baseline in only one of eight subjects after inhalation of unbuffered HCl and in no subjects after inhalation of unbuffered $H_2SO_4$, even at pH 2. In contrast, $SR_{aw}$ increased by greater than 50% in all eight subjects after inhalation of HCl and glycine at pH 2 and in seven of eight subjects after inhalation of $H_2SO_4$ and glycine at pH 2. The mean titratable acidity required to increase $SR_{aw}$ by 50% above baseline was calculated for each challenge by log-linear interpolation; the values for buffered $H_2SO_4$ (5.1 mL of 1N NaOH) and buffered HCl (2.2 mL of 1N NaOH) were slightly, but significantly different ($p < 0.01$) and were considerably higher than the titratable acidity of the unbuffered acids at pH 2 (1.0 mL of 1N NaOH) which did not cause much bronchoconstriction. The results of this study suggest that the acidity of inhaled large particle fogs can itself be a stimulus to bronchoconstriction. The bronchoconstrictor potency of acid fogs appears to be related to their total available hydrogen ion concentration (titratable acidity) and not merely to their free hydrogen ion concentration (pH) since, at a constant pH (pH 2), increasing amounts of titratable acidity caused increasing severity of bronchoconstriction for the two chemically distinct acid fogs studied. The greater potency of HCl compared to $H_2SO_4$ per unit of titratable acidity that was observed with the glycine-buffered solutions may have been due to the higher vapor pressure of HCl, which could have allowed vapor loss from the droplets and hence penetration of HCl vapor into the distal airways.

Because the conditions of exposure of this study [isoosmolar particles of relatively uniform diameter (MMAD 5–6 μm) delivered at a high concentration through a mouthpiece] were quite different from those encountered in the environment, it is not possible to extrapolate directly from our results to predict the effects of environmental exposure to acid fogs. Nonetheless, we were impressed by how weak a bronchoconstrictor stimulus unbuffered acid fogs were under the conditions we studied. Because titratable acidity appears to be a more important stimulus to bronchoconstriction than is pH, atmospheric monitoring during episodes of natural (or experimental) acid fog should include measurement of coexistent buffers and/or titratable acidity in addition to measurement of pH.

## Roles of pH and Ionic Species in Sulfur Dioxide- and Sulfite-Induced Bronchoconstriction

$SO_2$ and sulfites are well-described inducers of bronchoconstriction in individuals with asthma and are chemically related and, therefore, may share a common mechanism of action. When dissolved in aqueous solution, such as in the airway lining fluid, these sulfur oxide species enter into equilibrium with one another. $SO_2$ and metabisulfite convert to bisulfite ($HSO_3$) (pKa 1.86 and 0.09, respectively) and $HSO_3$, in turn, enters into equilibrium with sulfite ion ($SO_3$) (pKa 7.2). These reactions are accompanied by the release of hydrogen ions ($H^+$). We hypothesized that inhaled $SO_2$ induces bronchoconstriction through one of three possible mechanisms: (a) the formation of sulfites by the dissolving of $SO_2$ in water; (b) the entry of $SO_2$ itself into biochemical reactions; or (c) the liberation of $H^+$ by the dissolving of $SO_2$ in water. Additionally, it is possible that one of the sulfites might be more active than the others in causing bronchoconstriction.

To test these possibilities, we challenged 10 nonsmoking subjects with mild asthma with aerosols of sodium sulfite ($Na_2SO_3$) or acetic acid at various pHs and with $SO_2$ gas. We administered nebulized $Na_2SO_3$ solutions at pH 9 (containing 95% $SO_3$), at pH 6.6 (containing 80% $HSO_3$), and at pH 4 (containing 99% $HSO_3$ but greater than an order of magnitude more $SO_2$ than the pH 6.6 solution). Subjects inhaled increasing concentrations of aerosolized $Na_2SO_3$ at each pH during 1 min of tidal breathing. Subjects also inhaled buffered acetic acid aerosols with the same acidity as the pH 4 $Na_2SO_3$ solutions to control for the airway effects of acid aerosols. To assess sensitivity to $SO_2$ gas, subjects inhaled increasing concentrations of $SO_2$ during eucapneic hyperpnea. Bronchoconstriction was assessed by measurement of $SR_{aw}$ before and after each challenge.

Again, the subjects' asthma remained stable throughout the study, and there were no significant deviations in baseline $SR_{aw}$. Nine of the 10 subjects developed bronchoconstriction after inhaling the

$Na_2SO_3$ aerosols at all three pHs and the $SO_2$ gas. The mean concentration of $Na_2SO_3$ solution calculated to increase $SR_{aw}$ by 100% above baseline was significantly different ($p < 0.01$) at the various pHs: pH 4 (0.17 mg/mL) < pH 6.6 (0.49 mg/mL) < pH 9 (2.10 mg/mL). Only one subject responded to the acetic acid aerosol.

The results of this study confirm the reports of other investigators that inhaled $SO_3$ aerosols are a stimulus to bronchoconstriction in subjects with asthma (*5–7*). This effect is not restricted to individuals with a clinical history of $SO_3$ sensitivity because none of our subjects had such a history. The bronchoconstrictor potency of $Na_2SO_3$ aerosols was clearly pH dependent, with the greatest effect occurring at the most acid pH and the least effect at alkaline pH. However, acidity itself did not appear to be the stimulus to bronchoconstriction because most subjects were unaffected by inhalation of acetic acid with a titratable acidity many times greater than that contained in the concentration of $SO_3$ at pH 4 required to produce bronchoconstriction. Rather than exerting a direct effect, decreasing pH most likely increased $Na_2SO_3$-induced bronchoconstriction by altering the relative concentrations of $SO_3$, $HSO_3$, and $SO_2$ gas.

As bronchoconstriction occurred in 9 of 10 subjects after inhalation of concentrations of $Na_2SO_3$ at pH 9 not associated with measurable generation of $SO_2$ gas, it appears that sulfite species are themselves capable of inducing bronchoconstriction. Although at pH 9 there may have been some oxidation of $SO_3$ to sulfate ($SO_4$), the absolute magnitude of this conversion would have been small since the rate coefficient for this reaction ($3 \times 10^{-3}$/sec) corresponds to an $SO_3$ lifetime approximately 1000 times the residence time of $SO_3$ aerosol in our system (*8*). Because the airways are lined with water and $SO_2$ is rapidly converted to sulfites in an aqueous environment, it is possible that $HSO_3^-$ is the primary species responsible for $SO_2$-induced bronchoconstriction. In addition, stable inorganic sulfite species have been found in plumes and effluents from power plants and smelters (*9–11*). While the artificial conditions and high $SO_3$ concentrations employed do not allow extrapolation to such environmental exposures, the results of this study suggest that $SO_3$-containing aerosols could be stimuli to bronchoconstriction, especially when inhaled at acid pH.

## Acidity Potentiates Bronchoconstriction Induced by Hypoosmolar Fogs

Naturally occurring fogs are usually hypoosmolar with respect to body fluids (including airway lining fluid). Inhalation of hypoosmolar aerosols is well established as a potent stimulus to bronchoconstriction (*12–15*). Thus, we thought it would be important to characterize the nature of the interaction, if any, between hypoosmolarity and acidity in causing bronchoconstriction in subjects with asthma. Because of the limited bronchoconstrictor effects of unbuffered acid fogs demonstrated in the initial study described previously (*3*), we hypothesized that acidity would be more likely to potentiate the bronchoconstriction induced by hypoosmolarity than to have a significant independent effect.

To test this hypothesis, we studied in 12 nonsmoking subjects with mild asthma the bronchoconstrictor effects of fogs that varied with regard to both their osmolarity and acidity. We administered the following fogs: hypoosmolar saline (30 mOsm) at pH 5.5; three hypoosmolar acids ($H_2SO_4$, $HNO_3$, and a 1:1 mixture of $H_2SO_4$ and $HNO_3$, all 30 mOsm) at pH 2; and isoosmolar $H_2SO_4$ (300 mOsm) at pH 2. Because the airstream was fully saturated with water and the generated fogs were dense, off-gassing of nitric acid vapor was negligible (*16,17*). Each fog was administered on a separate day and was inhaled through a mouthpiece during tidal breathing. $SR_{aw}$ was measured before and after the subjects inhaled fog from an ultrasonic nebulizer for 3 min in up to five doubling nebulizer outputs.

Again, the subjects' asthma remained stable throughout the study and there were no significant deviations in baseline $SR_{aw}$. For each fog challenge, an output-response curve was generated and the nebulizer output required to increase $SR_{aw}$ by 100% above baseline ($PO_{100}$) was calculated. Mean values of $PO_{100}$ were significantly lower for each of the hypoosmolar acids than for hypoosmolar saline (mean ± SEM): 1.65 ± 0.43 g/min for saline compared to 0.95 ± 0.11, 1.05 ± 0.20 and 0.90 ± 0.14 for $H_2SO_4$, $HNO_3$, and a 1:1 mixture of the two acids, all $p$ values < 0.025. Mean values of $PO_{100}$ did not differ among the three acids studied. For 7 of 12 subjects, all three acids caused a clearcut leftward shift in the output-response curve from the curve generated for hypoosmolar saline fog. Isoosmolar $H_2SO_4$ did not increase $SR_{aw}$ by 100% in any subjects, even at the maximal nebulizer output, which delivered a concentration of $H_2SO_4$ in excess of 40 mg/m$^3$.

The results of this study suggest that acidity can significantly potentiate the bronchoconstriction caused by inhalation of a hypoosmolar fog in subjects with asthma. Since each of the three hypoosmolar, acidic solutions studied ($H_2SO_4$, $HNO_3$, or a 1:1 mixture of the two acids) had an equivalent bronchoconstriction-potentiating effect, the specific chemical composition of the solution did not appear to be an important factor. As we reported in the initial study described above, large particle aerosols (MMAD 5–6 microns) of unbuffered isoosmolar $H_2SO_4$ caused little bronchoconstriction.

The conditions of exposure we studied were quite different from those encountered in the environment. The liquid water content of the fogs (ranging from 6 to 87 g/m$^3$) was many times higher than that which has been measured during even worst case natural fog

conditions (2 g/m$^3$) (*18*). In addition, the $H_2SO_4$ concentrations we studied were many times higher than those encountered in natural fog. Despite the high concentrations of water and $H_2SO_4$ used in this study, our results might be relevant to possible adverse health effects of acid fog. The exposures used were brief (3 min) and occurred during resting tidal breathing. It is possible that longer exposures, especially during exercise, might lead to significant bronchoconstriction during exposure to fogs with lower water content and/or acid concentrations. In any case, the results of this study suggest that the interaction of acidity and osmolarity needs to be considered in the design and interpretation of studies of the respiratory health effects of acid fog.

The studies summarized contribute to the characterization of the bronchoconstrictor potency of several stimuli present in acid fogs in individuals with asthma. Titratable acidity appears to be a more important stimulus to bronchoconstriction than is pH, and unbuffered acid fogs have only weak bronchoconstrictor effects. However, unbuffered acidity can potentiate the bronchoconstriction caused by inhalation of a hypoosmolar fog. Finally, sulfite species are themselves capable of inducing bronchoconstriction, especially when inhaled at acid pH and $HSO_3^-$ may be the primary species responsible for $SO_2$-induced bronchoconstriction.

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